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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	UF-232XC1
First Inventor or Application Identifier	Jane E. Polston
Title	Materials and Methods for Producing Geminivirus Resistant Plants
Express Mail Label No.	EK318905915US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)* (3 pages)
   (Submit an original and a duplicate for fee processing)
2. [X] Specification [Total Pages 21]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 U.S.C. 113)* [Total Sheets 3]
4. Oath or Declaration [Total Pages 2]
   a. [X] Newly executed (original or copy) (unsigned)
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).

5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [X] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
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Applicant or Patentee: Jane E. Polston, Ernest Hiebert, Ahmed M. Abouzid, Wayne B. Hunter
Attorney's Docket No. UF-232XC1
Serial or Patent No.:
Filed or Issued: January 25, 2000
For: Materials and Methods for Producing Geminivirus Resistant Plants

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (c)) – NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION University of Florida
ADDRESS OF ORGANIZATION 223 Grinter Hall
Gainesville, FL 32611

TYPE OF ORGANIZATION

[X] UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
[ ] TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a)(3))
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I hereby declare that the above identified nonprofit organization qualifies as a nonprofit organization as defined in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, with regard to the invention described in the above-identified:

[ ] PATENT [x] APPLICATION

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If the rights held by the above identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring their status as small entities. (37 CFR 1.27)

NAME _____
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I acknowledge the duty to file, in this application or patent, notification of any change of status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Thomas E. Walsh, Ph.D.
TITLE IN ORGANIZATION Director of Sponsored Research
ADDRESS OF PERSON SIGNING 223 Grinter Hall
Gainesville, FL 32611

SIGNATURE TWalsh DATE January 25, 2000

DESCRIPTION

MATERIALS AND METHODS FOR PRODUCING GEMINIVIRUS RESISTANT PLANTS

The subject invention was made with government support under a research project supported by USDA Grant No. 92341357456 and USDA Grant No. 98341356784. The government has certain rights in this invention.

Cross-Reference to a Related Application

This application claims the benefit of U.S. Provisional Application No. 60/117,151 filed January 25, 1999.

Background of the Invention

Whitefly-transmitted geminiviruses have become a major limiting factor in tomato production in Florida, the Caribbean and much of Latin America. This group of viruses is currently expanding in the Western Hemisphere, and the number of characterized geminiviruses which infect tomato in this region has increased from three to more than 17 over the last 10 years (Polston and Anderson, 1997). This expansion is continuing and reports of new epidemics are appearing almost monthly. Whitefly-transmitted viruses appear alone and in mixed infections with other geminiviruses and other viruses. Whitefly-transmitted geminiviruses are reducing tomato yields in many countries, and total crop losses are not uncommon (Polston and Anderson, 1997). Tomato production in Florida has suffered significant losses (estimated at $125 million in 1990-91) due to tomato mottle virus (ToMoV) infection, which first appeared in 1989. There are no estimates of losses in Puerto Rico due to the tomato geminiviruses, potato yellow mosaic virus (PYMV) and ToMoV, but yields have been reduced significantly (Brown *et al.*, 1995). Tomato yellow leaf curl virus (TYLCV-Is) which caused extensive losses to tomato production in the Dominican Republic

(reviewed by Polston and Anderson, 1997) has now been found in Florida (Polston *et al.*, 1999). Incidences of TYLCV-Is are increasing and economic losses were felt this past fall (1998). TYLCV-Is is widespread in Florida, is likely to increase over the next few years and will become a major constraint to tomato production in Florida.

Geminiviruses are very difficult to economically manage in fresh market tomatoes, and practically impossible to manage in processing tomatoes. At this time geminiviruses are managed primarily through the use of a single insecticide, imidacloprid, to reduce the population of the whitefly vector. Tolerance to this insecticide has already been reported from other countries (Cahill *et al.*, 1996; Williams *et al.*, 1996). It may be only a matter of time before imidacloprid loses efficacy in the United States and other locations. The average Florida tomato grower spent approximately $250/acre for insecticides to control ToMoV in 1994 through 1997. These costs are expected to increase significantly as growers' struggle to manage TYLCV-Is. In Caribbean countries geminiviruses have caused many small and medium size tomato growers to go out of business due the increases in costs of production and crop losses. In Israel, where imidacloprid resistance is present, TYLCV-Is is managed by pesticide use plus exclusion; tomatoes are produced in greenhouses enclosed in whitefly-proof screening material or in screened tunnels in the field. The use of these methods are expensive and are often not an economically or horticulturally realistic alternative. The least expensive and most practical control of whitefly-transmitted geminiviruses is the use of resistant cultivars. At this time there are no commercially available resistant tomato cultivars for the geminiviruses native to the Western Hemisphere. There are several cultivars available which have tolerance to TYLCV-Is, however the fruit size and the horticultural attributes of these cultivars are unsuitable for production in Florida.

There are no commercially available ToMoV-resistant tomato cultivars. ToMoV-resistance from *Lycopersicon* species has been incorporated into tomato (*L. esculentum*) backgrounds but resistance is closely linked with small fruit size. This linkage has significantly delayed development of resistant plants. Resistance to ToMoV in both tobacco and tomato has been described using mutated coat protein and movement protein genes from

ToMoV (Abouzid *et al.*, 1996; Duan *et al.*, 1997a; Duan *et al.*, 1997b; Polston *et al.*, 1996; Sinisterra *et al.*, 1997; Sinisterra *et al.*, 1999). A mutated *BC1* gene has been shown to give broad-spectrum resistance (Duan *et al.*, 1997a).

There are few reports suggesting that the gene encoding the geminivirus replication associated protein (Rep) might be used for resistance. There has been a report that a modified ToMoV *Rep* mutated in a NTP-binding motif was transformed into tomato plants and demonstrated to interfere with viral replication (Stout *et al.*, 1997). Hanson *et al.* (1995) analyzed phenotypes of BGMV (bean golden mosaic virus) with mutations in a NTP-binding motif of the *Rep* gene, and demonstrated that the NTP-binding domain is required for replication. They proposed that mutations in this motif may serve in a trans-dominant negative interference scheme for pathogen-derived resistance (also known as "dominant negative mutations"). Resistance to African cassava mosaic geminivirus (ACMV) in *Nicotiana benthamiana* plants was developed by transformation with ACMV *Rep* (Hong and Stanley, 1996).

Resistance has been reported with the *Rep* gene of a monopartite virus, tomato yellow leaf curl virus (TYLCV), a geminivirus only distantly related to ToMoV. Noris *et al.* (1996) found TYLCV-resistance in *N. benthamiana* plants using the TYLCV *Cl* gene with a truncated C-terminal (210 amino acids). However, resistance was overcome with time. Brunetti *et al.* (1997) transformed tomatoes with the same construct and found that high accumulation of the truncated Rep protein was required for resistance, that high accumulation resulted in a "curled" phenotype, and that the resistance did not extend to an unrelated geminivirus. The plants transformed according to the methods of the subject invention have a normal phenotype and are high yielding as well.

Brief Summary of the Invention

The subject invention pertains to materials and methods for producing plants that are resistant to infection by geminiviruses and other related viruses. Methods of the invention comprise transforming a plant with a polynucleotide wherein when the polynucleotide is

expressed in the plant, the transformed plant exhibits resistance to infection when challenged with a plant virus. In a preferred embodiment, a plant is transformed with a polynucleotide encoding a Rep protein or a mutated Rep protein derived from tomato mottle geminivirus or from tomato yellow leaf curl virus (TYLCV-Is). The methods of the invention can be used to provide resistance to viral infection in plants such as tomato and tobacco.

The subject invention also concerns polynucleotides that encode the Rep protein and mutated Rep proteins of the invention. The mutated Rep proteins are also an object of the present invention.

The present invention also concerns transformed and transgenic plants and plant tissue that contain or express a polynucleotide encoding a Rep protein or a mutated Rep protein.

Brief Description of the Drawings

Figures 1A and 1B show a Field Resistance Trial conducted in Fall 1997. **Figure 1A** shows disease progress curves of ToMoV in 'Agriset 761' and 6 tomato lines transformed with ToMoV *Rep* gene. **Figure 1B** shows the mean number of immature whiteflies per ten terminal leaflets.

Figures 2A and 2B show a Field Resistance Trial conducted in Spring, 1998. **Figure 2A** shows disease progress curves of ToMoV in 'Agriset 761', FL 7324, FL 7613 and 4 tomato lines transformed with ToMoV *Rep* gene. **Figure 2B** shows the mean number of immature whiteflies per ten terminal leaflets.

Figures 3A and 3B show a Field Resistance Trial conducted in Fall 1998. **Figure 3A** shows disease progress curves of ToMoV in 'Agriset 761', FL 7324, FL 7613, and 5 tomato lines transformed with ToMoV *Rep* gene. **Figure 3B** shows the mean number of immature whiteflies per ten terminal leaflets.

Detailed Description of the Invention

The subject invention concerns the use of a plant virus gene to transform a plant or plant tissue to confer resistance in the plant or plant tissue to infection from a plant virus. The methods of the subject invention can be used to confer resistance in a plant to infection by a plant pathogen such as, for example, a geminivirus. The method comprises transforming a plant with a polynucleotide such that when the polynucleotide is expressed in the plant the plant then exhibits resistance to infection by plant viruses. In one embodiment of the invention, a plant is transformed by wounding and agroinfection with an *Agrobacterium* containing a polynucleotide of the invention that is transferred to the plant upon agroinfection of the plant. Preferably, the polynucleotide used in the methods of the invention encodes a plant virus Rep protein or a mutant Rep protein, or a fragment or variant thereof. In an exemplified embodiment, the polynucleotide encodes a Rep protein of tomato mottle geminivirus (ToMoV). The nucleotide sequence of a ToMoV (component A) virus is disclosed in Genebank having accession number L14460. Abouzid *et al.* (1992) disclose the nucleotide sequence of the ToMoV *Rep* gene (referred to therein as AL1 and corresponding to nucleotides 1523 to 7 of the sequence shown in Figure 1 of Abouzid *et al.* (1992)). In another embodiment, the polynucleotide encodes a tomato yellow leaf curl virus (TYLCV-Is) Rep protein. The nucleotide sequences of several TYLCV-Is viral isolates are disclosed in Genebank, including isolates from Israel (accession number X15656), Cuba (accession number AJ223505), Dominican Republic (accession number AF024715), Egypt (accession number L12219), Jamaica (accession number U84146), Lebanon (accession number AF160875), Mexico (accession number AF168709) and Spain (accession number AJ223505).

In a preferred embodiment of the invention, a virus-resistant transgenic plant line prepared according to the methods described herein is crossed with a transgenic plant line that is resistant to the same virus and derived from a different transformation event to produce hybrids that exhibit increased virus resistance over the parent lines.

The methods of the subject invention can be used to confer resistance in plants to infection by viruses such as geminiviruses, and include, for example, tomato mottle virus, cabbage leaf curl geminivirus, potato yellow mosaic virus, tomato golden mosaic virus, tomato yellow mosaic virus, tomato leaf crumple virus, tomato yellow leaf curl virus, pepper huasteco virus and others. Plants which can be transformed according to the methods of the subject invention include, but are not limited to, tomato and tobacco.

The subject invention also concerns polynucleotide molecules that encode modified or mutated forms of a plant virus Rep protein which when expressed in a plant confers resistance to infection by plant viruses. In one embodiment, the polynucleotide encodes a Rep protein of ToMoV or TYLCV-Is. Modifications and mutations contemplated within the scope of the invention include Rep proteins comprising amino acid substitutions, deletions, and insertions. Also contemplated within the scope of the invention are Rep polypeptides containing the mutations in the amino acid sequence.

The subject invention also concerns recombinant polynucleotide molecules comprising a vector in which a polynucleotide sequence encoding a plant virus Rep protein, or a mutant thereof, which is expressible in a suitable host plant has been inserted. Suitable vectors may be selected from those known in the art including plasmids, phage DNA, or derivatives or fragments thereof, or combinations of plasmids and phage DNA, and yeast plasmids. The polynucleotide encoding the Rep protein can be inserted into the multiple cloning site of a vector, such as the commercially available pUC vectors or the pGEM vectors, which allow for the excision of the polynucleotide having restriction termini adapted for insertion into any desirable plant expression or integration vector. In addition, regulatory sequences such as promoters can be operatively linked to the coding sequences of the polynuceotides of the present invention. For example, the 35S promoter of cauliflower mosaic viruses (CaMV) can be used with the subject invention. Other plant expression vectors can also be used in the present invention.

The present invention also concerns cells infected, transformed, or transfected with a polynucleotide of the present invention that encodes a Rep protein or a mutated Rep

protein. Preferably, the Rep protein or mutant thereof is derived from ToMoV or TYLCV-Is. In one embodiment, the polynucleotide is inserted into a suitable vector, and the recombinant vector is used to transform a bacterium or other host which can then be used to introduce the polynucleotide into a plant cell. Suitable hosts that can be infected, transformed, or transfected with the polynucleotide of the invention include gram positive and gram negative bacteria such as *E. coli* and *Bacillus subtilis*. Other suitable hosts include *Agrobacterium* cells, insect cells, plant cells, and yeast cells. *Agrobacterium* containing the polynucleotide of the invention can be used to transform plant cells with the polynucleotide according to standard methods known in the art. Polynucleotides can also be introduced into plant cells by a biolistic method (Carrer, 1995) and other methods known in the art.

The subject invention also concerns transformed and transgenic plants and plant tissue, including plant seeds, that exhibit resistance to infection by plant geminiviruses such as ToMoV and the like. In one embodiment, a transformed or transgenic plant of the invention comprises a polynucleotide that encodes a Rep protein or a mutated Rep protein. Preferably, the Rep protein or mutated Rep protein is derived from ToMoV or TYLCV-Is. Transformed and transgenic plants and plant tissue of the invention can be prepared from plants such as tomato, tobacco and others.

As those of ordinary skill in the art will appreciate, any number of different nucleotide sequences can be used, based on the degeneracy of the genetic code, to encode a Rep protein or a mutated Rep protein of the present invention. Accordingly, any polynucleotide sequence which encodes a Rep protein or mutated Rep protein, or a fragment or variant thereof, falls within the scope of this invention.

Two hybrid parent tomato lines (from J.W. Scott) FL 7324 and FL 7613, were transformed with the ToMoV *Rep* gene in the sense orientation. Both tolerance and immunity to ToMoV were seen in plants containing the transgene in T_1 through T_4 generations. Preliminary Southern analysis has indicated that resistant plants have either one or two genes. Resistance has been evaluated in the field in the fall and spring seasons of 1996, 1997, and 1998. Plants in the field were selected for resistance and horticultural

qualities. Yields of transformed plants were equivalent to non-transformed plants in the absence of virus, and were significantly greater in the presence of ToMoV. Transformed plants appeared to have high levels of tolerance to ToMoV.

Resistance to infection was evaluated by simulating natural inoculation as much as possible. Other laboratories use such techniques as biolistic and Agro-inoculation, which never occur naturally, and bypass the normal modes of entry into the plant cell where resistance mechanisms may exist. The inoculation described herein is a simulation of a worst case scenario in a transplant house or a grower's field. Plants are inoculated at an early stage in development, when plants are highly attractive to whiteflies and are highly susceptible to infection by ToMoV. Whiteflies are reared on virus-infected tomato plants, which eliminates the interference of whitefly feeding preferences, and is similar to inoculation by viruliferous whiteflies in the field (Polston *et al.*, 1996). This inoculation protocol results in an inoculation efficiency of 100%.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 — Field Evaluation of Transgenic Resistance to ToMoV

Advanced breeding lines, FL 7324 and FL 7613 were transformed with ToMoV *Rep* gene using standard *Agrobacterium*-mediated transformation techniques. A vector comprising a 35S CaMV (non-enhanced) promoter linked to the *Rep* gene was used in the transformation. Plant tissue was wounded using tungsten. Plants that contained a *Rep* transgene were identified using PCR methods. Those plants were then evaluated for resistance to viral infection. Untransformed parents and transformed lines from three

different transformation events were evaluated for resistance to ToMoV and for yield, both in the presence and absence of ToMoV. Lines shown in the following tables are four generations past transformation. Lines 02 and 04 are from the same T_0 plant in a FL 7613 background, lines 09 and 10 are from a second T_0 plant and their background is FL 7324, and lines 11 and 12 are from a third T_0 plant, and their background is FL 7613.

Southern analysis using two restriction enzymes, one which cut inside the transgene and one which cut outside the transgene, of several lines of Rep-transformed tomatoes revealed that the ToMoV resistance in 4 of the R4 generation lines (lines 02, 04, 11, 12) appeared to be due to one insertion site and one copy of the transgene. Multiple copies were present in resistant lines 09 and 10. Lines 02 and 04, 11 and 12, and 09 and 10 were the result of three different transformations.

Example 2 — Performance in the Presence of ToMoV and Whiteflies

For three seasons, Fall 1997, Spring 1998, and Fall 1998, tomato transplants to be evaluated were set into a field which was within 20 feet of a large block of tomatoes which was a continuous source of viruliferous whiteflies throughout the season. No imidacloprid was applied to the plants being evaluated but attempts were made to keep whitefly populations below a threshold which would result in irregular ripening of the fruit (20 immature whitefly/10 terminal leaflets). Whitefly populations were evaluated approximately every 2 week beginning about 4 week after transplanting. Whitefly populations varied each season, with the highest populations occurring in the Fall 1998 trial. The trials consisted of 15 plants per block, with three replications, in a randomized complete block design. Plants were evaluated every other week for the presence of whiteflies and virus. Plants displaying virus-like symptoms were assayed by nucleic acid hybridization to confirm the presence of ToMoV. Fruit were harvested from plants in two pickings, graded, and marketable yields were calculated.

Example 3 — Yields

Results are shown in Tables 1, 2 and 3. The transformed lines yielded as much or more than the untransformed parents and the commercial hybrid 'Agriset' in all three trials. The best transformed lines, 02, 04, 11 and 12 yielded approximately 50% - 100% more marketable fruit than the untransformed lines. Yields of these transformed lines in the presence of ToMoV and whiteflies were comparable to yields of the untransformed lines in the absence of virus and whiteflies. In addition, transformed plants yielded well in both fall and spring production seasons.

Example 4 — ToMoV Resistance

Infection rates as determined by viral nucleic acid detection, were much lower in all transformed lines than in untransformed lines. Transformed lines has high levels of tolerance, which were overcome only with high populations of viruliferous whiteflies. Figures 1A, 2A, and 3A show the disease progress curves from untransformed and transformed lines from trials over three seasons. The highest rates of infection were observed in the Fall 1998 season (Figures 3A and 3B) which had extremely high populations of viruliferous whiteflies (at 100 per 10 terminal leaflets).

Even with those unusually high populations, transformed lines though infected produced yields similar to those plants not exposed to virus (Tables 3 and 6). Symptoms in infected transformed plants were milder than those of infected untransformed plants.

Example 5 — Performance in the absence of ToMoV and Whiteflies

For three seasons, Fall 1997, Spring 1998, and Fall 1998, tomato transplants to be evaluated were set into a field which was not located near a source of ToMoV or whiteflies. Imidacloprid was applied at the time of transplant to the field, and plants were monitored weekly for whiteflies. When whiteflies were detected (about the 6 to 8 week after transplant) plants were sprayed with a rotation of insecticides to manage whitefly populations. This resulted in less than 0.1% infection of ToMoV in these plants, and allowed an evaluation of

yields without the influence of virus. The trials consisted of 15 plants per block, with three replications, in a randomized complete block design. Plants displaying virus-like symptoms were assayed by nucleic acid hybridization to confirm the presence of ToMoV. Fruit were harvested from plants in two pickings, graded, and marketable yields were calculated.

Example 6 — Yield

Marketable yields of untransformed and transformed plants are shown in Tables 4, 5, and 6. Yields of transformed lines were either significantly greater (Table 4) or not significantly different to those of the transformed plants. The best yielding transformed lines were 02, 04, 11 and 12 which yielded as good or better than their untransformed parent, FL 7613 in the absence of ToMoV infection.

Example 7 — Hybrid Transgenic Tomatoes

Hybrid tomatoes were made by crossing transgenic lines with the untransformed genotype and between transgenic lines derived from different transformation events. It was found that several of the hybrids of different transgenic lines were more resistant to ToMoV than either open-pollinated parent. This is known as pyramiding of resistance genes and resulted in improved resistance of the transgenic plants to infection.

Transgenic lines 02 and 11 were crossed and their hybrid progeny was evaluated for yield and ToMoV resistance. An increase in resistance was observed in the hybrid. ToMoV resistance in the hybrids was superior to both the transformed parents and nontransformed parents (Table 7). Infection of the hybrid was 1/3 that of the transgenic parent and 1/10 that of the untransformed parent. Resistance appeared to be additive. Yields of these crosses are currently being analyzed but are expected to be high based on previous results with the untransformed parents. This data shows that hybridizing transgenic parents is a method to improve geminivirus resistance.

Tables 1-3 show a comparison of yields of ToMoV *Rep* - transformed tomatoes with untransformed parents and 'Agriset' in the presence of ToMoV and whiteflies.

Tables 4-6 show a comparison of yields of ToMoV *Rep* - transformed tomatoes with untransformed parents in the absence of ToMoV and whiteflies.

Table 1. Fall 1997 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1556.5 a	766.7 a	0.33 ab	580.8 a
04	1695.9 a	952.5 a	0.38 a	580.8 a
09	952.5 b	92.9 c	0.27 c	511.1 a
10	859.6 b	69.7 c	0.28 c	464.6 a
FL 7324	-	-	-	-
FL 7613	-	-	-	-
Agriset	952.5 b	325.2 b	0.31 bc	325.2 b

Table 2. Spring 1998 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1498.5 a	1028.9 a	0.394 a	627.3 a
04	1237.1 ab	923.5 a	0.373 a	697.0 a
09	1359.1 ab	156.8 b	0.266 bc	592.4 a
10	1341.6 ab	174.2 b	0.261 c	609.8 a
FL 7324	906.1 ab	139.4 b	0.272 c	592.4 a
FL 7613	784.1 b	487.9 b	0.365 a	348.5 a
Agriset	714.4 b	278.8 b	0.320 b	331.1 a

Table 3. Fall 1998 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls[1] (ca/A)
04	1702.9 a[2]	1247.6 a	0.377 a	480.0 ab
10	759.7 b	30.2 b	0.240 d	736.5 a
11	1689.0 a	999.0 a	0.358 ab	573.8 ab
12	1905.0 a	1191.8 a	0.356 ab	573.8 ab
FL 7324	325.2 b	23.2 b	0.284 cd	401.9 b
FL 7613	727.2 b	464.6 b	0.377 ab	471.6 ab
Agriset	580.8 b	255.6 b	0.323 bc	325.3 b

[1]Culls include all fruit rated not marketable (includes insect damage, disease, irregular shape, *etc.*)

[2] Letters after values denote significant differences as determined by Duncan's Multiple Range.

Table 4. Fall 1997 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1359.1 a	731.8 a	0.35 ab	609.8 a
04	1184.8 ab	662.1 a	0.35 ab	592.4 a
09	906.1 bc	104.5 c	0.27 c	400.8 a
10	609.8 cd	22.7 c	0.26 c	435.6 ab
FL 7324	993.2 b	278.8 bc	0.32 b	313.6 b
FL 7613	592.4 d	435.6 ab	0.37 a	278.8 b

Table 5. Spring 1998 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls (ca/A)
02	1968.9 a	1219.7 ab	0.36 b	784.1 a
04	2108.3 a	1515.9 ab	0.37 b	784.1 a
09	1986.3 a	296.2 c	0.29 c	540.1 ab
10	2265.1 a	278.8 c	0.26 d	418.2 ab
FL 7324	2456.8 a	906.0 bc	0.30 a	278.8 b
FL 7613	2317.4 a	1812.1 a	0.40 a	435.6 ab
Agriset	2352.2 a	1550.7 ab	0.36 b	313.6 b

Table 6. Fall 1998 Trial				
Line	Total Marketable Yield (ca/A)	Extra Large Fruit (ca/A)	Avg. Fruit Size (lbs)	Culls[1] (ca/A)
02	1568.2 abc[2]	911.3 b	0.348 b	412.9 a
04	1503.7 abc	1050.7 b	0.368 b	423.4 a
09	744.0 d	12.2 c	0.240 d	639.5 a
10	998.4 cd	64.5 c	0.272 c	557.6 a
11	1747.6 ab	1115.1 ab	0.351 b	597.6 a
12	1742.4 ab	1197.0 ab	0.361 ab	522.7 a
FL 7324	1184.8 bcd	174.2 c	0.270 c	418.2 a
FL 7613	1951.5 a	1510.7 a	0.388 a	418.2 a
Agriset	1381.7 abcd	876.4 d	0.360 ab	505.3 a

[1]Culls include all fruit rated not marketable (includes insect damage, disease, irregular shape, etc.)

[2] Letters after values denote significant differences as determined by Duncan's Multiple Range.

Table 7. Evaluation of Resistance to ToMoV in a Hybrid of Two Transgenic Lines - Fall 1998 Trial		
Line	Transformation Status	Incidence of ToMoV (60 days post transp.)[1]
FL 7324	not transformed	100%
FL 7613	not transformed	100%
F97/02	FL 7613- Rep	36.7%
F97/11	FL 7324 - Rep	33.3%
F_1	F97/0202 x F97/11	12%

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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Claims

We claim:

1. A method for providing resistance to infection by a plant virus in a plant or plant tissue, said method comprising transforming said plant or plant tissue with a polynucleotide that encodes a Rep protein, or a fragment or variant thereof, of said plant virus.

2. The method according to claim 1, wherein said plant virus is a geminivirus.

3. The method according to claim 1, wherein said geminivirus is selected from the group consisting of tomato mottle virus, cabbage leaf curl geminivirus, potato yellow mosaic virus, tomato golden mosaic virus, tomato yellow mosaic virus, tomato leaf crumple virus, tomato yellow leaf curl virus and pepper huasteco virus.

4. The method according to claim 1, wherein said polynucleotide encodes a Rep protein of a tomato mottle geminivirus.

5. The method according to claim 1, wherein said polynucleotide encodes a Rep protein of a tomato yellow leaf curl virus (TYLCV-Is).

6. The method according to claim 1, wherein said plant or plant tissue is tomato or tobacco.

7. The method according to claim 1, wherein said plant or plant tissue is transformed with said polynucleotide by agroinfection.

8. The method according to claim 1, wherein said plant or plant tissue is transformed with said polynucleotide by biolistic targeting.

9. A transgenic plant or plant tissue having increased resistance to infection by a plant virus, wherein said plant or plant tissue comprises a polynucleotide sequence that encodes a plant virus Rep protein, or a fragment or variant thereof.

10. The transgenic plant or plant tissue according to claim 9, wherein said plant or plant tissue is tomato or tobacco.

11. The transgenic plant or plant tissue according to claim 9, wherein said polynucleotide encodes a Rep protein of a tomato mottle virus.

12. The transgenic plant or plant tissue according to claim 9, wherein said polynucleotide encodes a Rep protein of a tomato yellow leaf curl virus (TYLCV-Is).

13. The transgenic plant or plant tissue according to claim 9, wherein said plant tissue is a plant seed.

14. The transgenic plant or plant tissue according to claim 9, wherein said transgenic plant or plant tissue is crossed with a second transgenic plant or plant tissue that is resistant to said plant virus and derived from a distinct transformation event, producing a hybrid plant or plant tissue that exhibits increased resistance to infection by said plant virus.

15. A cell transformed with a polynucleotide sequence that encodes a plant virus Rep protein, or a fragment or variant thereof.

16. The transformed cell according to claim 15, wherein said polynucleotide encodes a Rep protein of a tomato mottle virus.

17. The transformed cell according to claim 15, wherein said polynucleotide encodes a Rep protein of a tomato yellow leaf curl virus (TYLCV-Is).

18. The transformed cell according to claim 15, wherein said cell is selected from the group consisting of bacterial cell, insect cell, plant cell and yeast cell.

Abstract of the Disclosure

The subject invention pertains to materials and methods for producing plants that are resistant to infection by geminiviruses and other related viruses. Methods of the invention comprise transforming a plant with a polynucleotide wherein when the polynucleotide is expressed in the plant, the transformed plant exhibits resistance to plant viral infections. Exemplified herein is the use of a polynucleotide encoding a Rep protein derived from tomato mottle geminivirus. The methods of the invention can be used to provide resistance to viral infection in plants such as tomato and tobacco. The present invention also concerns transformed and transgenic plants in plant tissue that express a polynucleotide encoding a plant virus Rep protein, or a fragment or variant thereof.

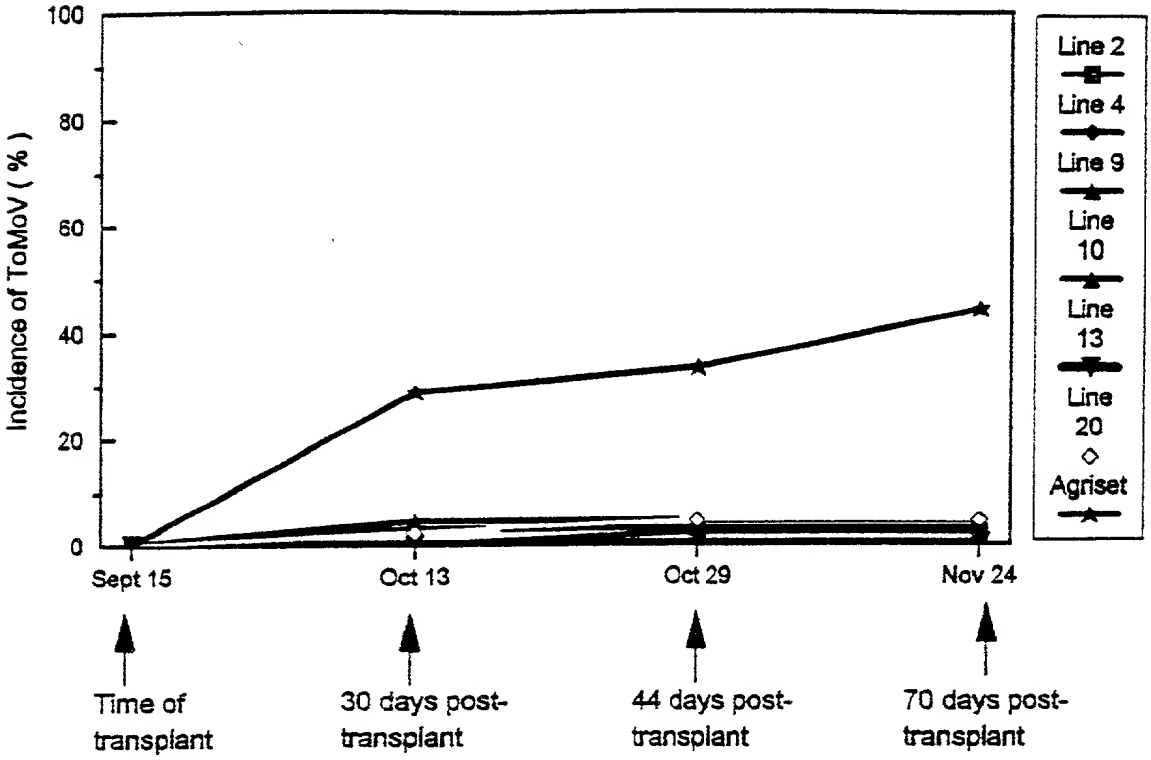

100
80
60
40
20
0
Incidence of ToMoV (%)
Line 2
Line 4
Line 9
Line 10
Line 13
Line 20
Agriset
Sept 15
Oct 13
Oct 29
Nov 24
Time of transplant
30 days post-transplant
44 days post-transplant
70 days post-transplant

FIG. 1A

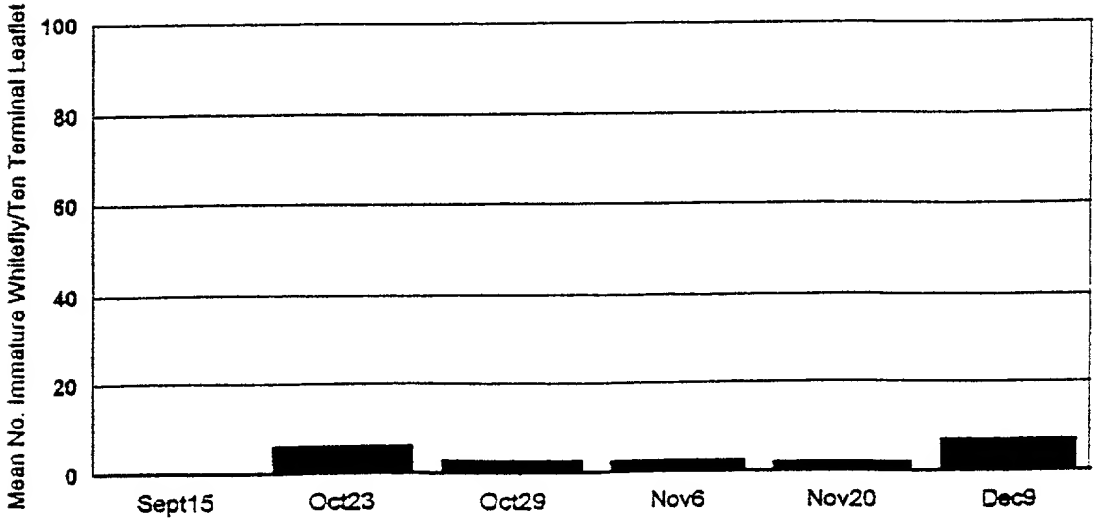

Mean No. Immature Whitefly/Ten Terminal Leaflet
100
80
60
40
20
0
Sept15
Oct23
Oct29
Nov6
Nov20
Dec9
Mean Number of Immature Whiteflies per Ten Terminal Leaflets

FIG. 1B

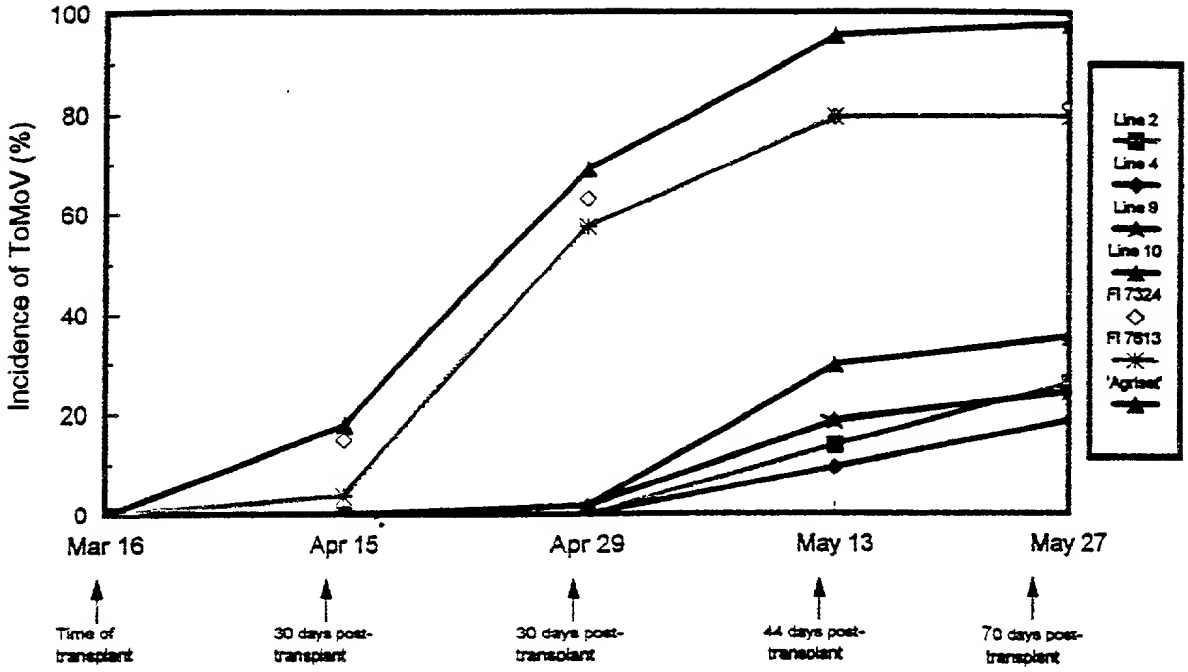
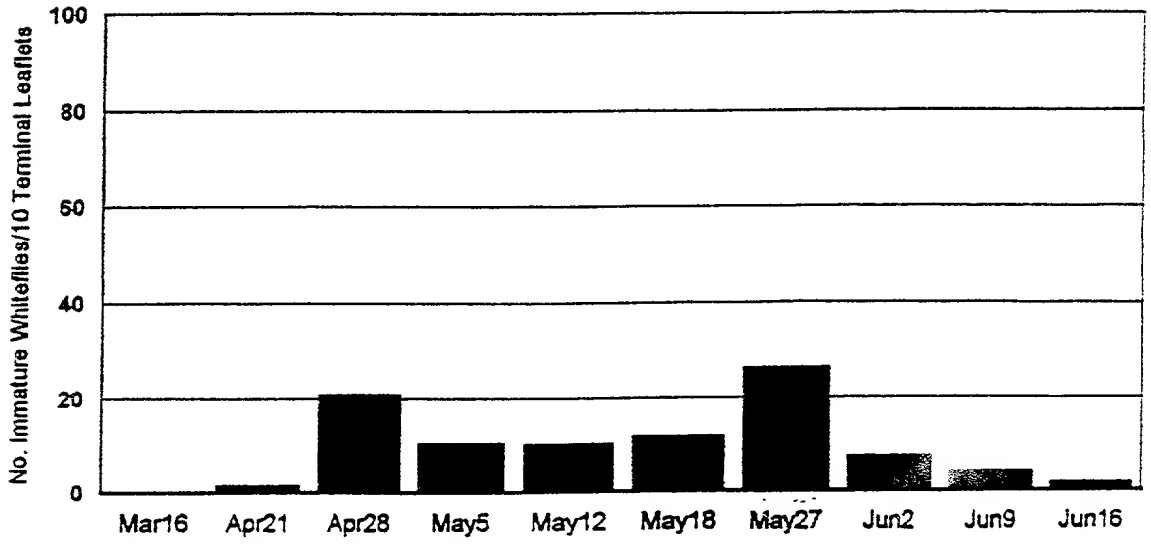


FIG. 2A



Mean Number of Immature Whiteflies per Ten Terminal Leaflets

FIG. 2B

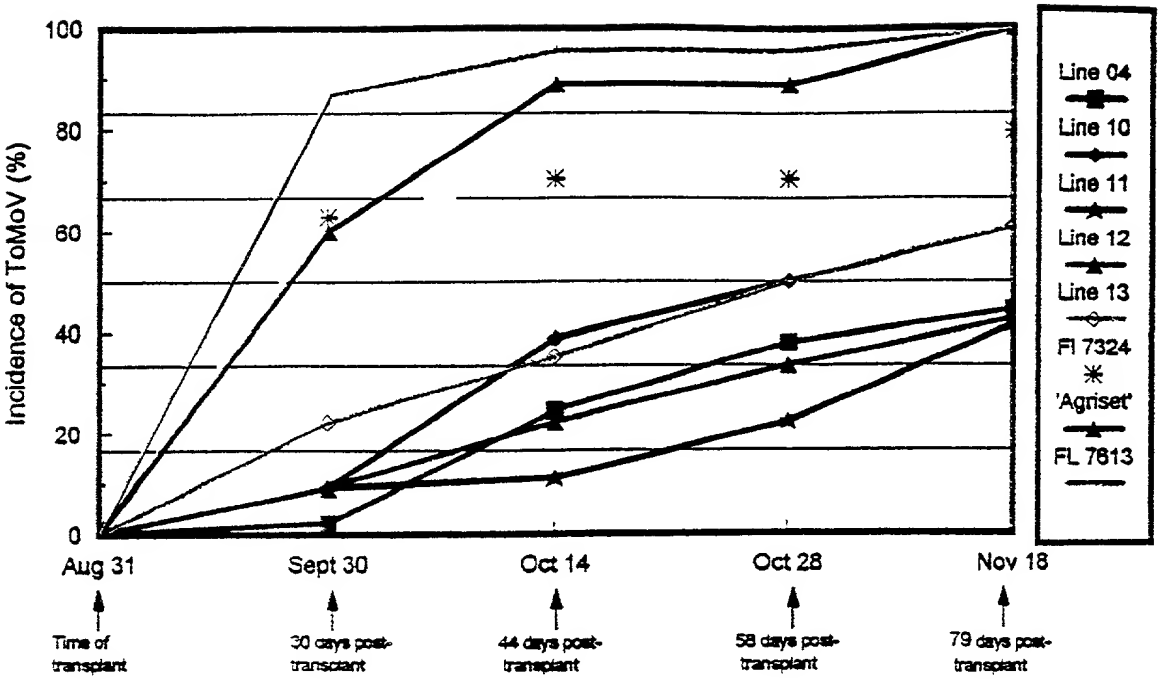
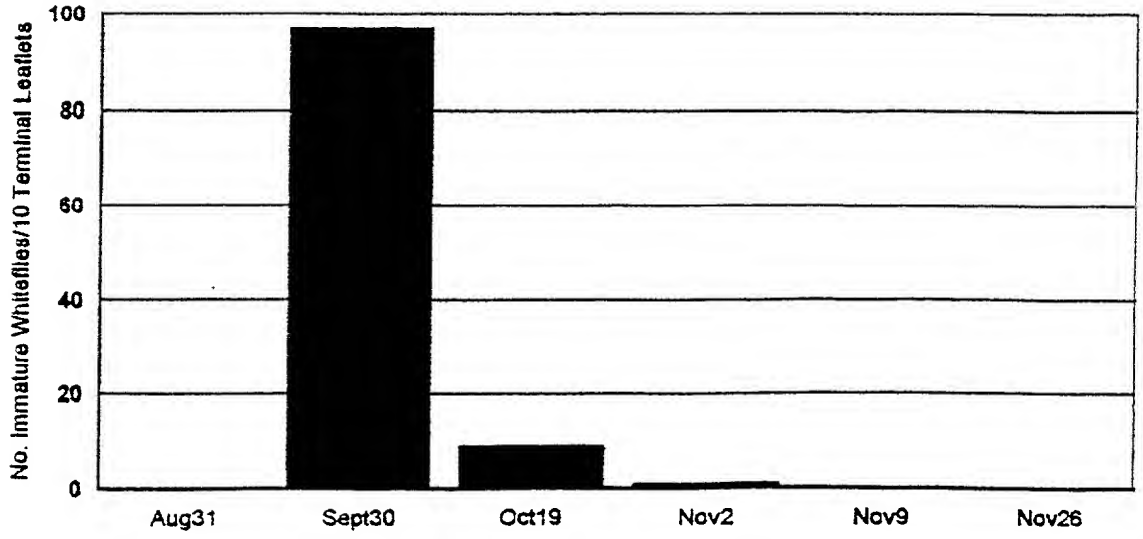


FIG. 3A



Mean Number of Immature Whiteflies per Ten Terminal Leaflets

FIG. 3B

Docket No. UF-232XC1

DECLARATION (37 CFR 1.63) AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name; and

I believe that I am the original, first, and sole inventor (if only one name is listed below), or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **MATERIALS AND METHODS FOR PRODUCING GEMINIVIRUS RESISTANT PLANTS** the specification for which

☒ is attached hereto.

☐ was filed _____, Serial No. _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application Serial No.	Country	Filing Date	Priority Claimed

I hereby claim priority benefits under Title 35, United States Code §119 of any provisional application(s) for patent listed below:

Application Serial No.	Filing Date	Priority Claimed
60/117,151	January 25, 1999	Yes

I hereby claim the benefit under Title 35, United States Code, §120 and/or §365 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following persons registered to practice before the Patent and Trademark Office as my attorneys with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith: David R. Saliwanchik, Reg. No. 31,794; Jeff Lloyd, Reg. No. 35,589; Doran R. Pace, Reg. No. 38,261; Christine Q. McLeod, Reg. No. 36,213; Jay M. Sanders, Reg. No. 39,355; James S. Parker, Reg. No. 40,119; Jean Kyle, Reg. No. 36,987; Frank C. Eisenschenk, Reg. No. 45,332; Seth M. Blum, Reg. No. P-45,489.

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